Carboxy Ester Hydrolysis Promoted by a Dicopper(II) Macrocyclic Polyamine Complex with Hydroxypropyl Pendant Groups

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A dinuclear Cu^{II} complex containing a hexaaza macrocyclic ligand bearing two 2-hydroxypropyl pendants, 3,6,9,16,19,22-hexaaza-6,19-bis(2-hydroxypropyl)tricyclo-$[22.2.2.2^{11,14}]$triaconta-1,11,13,24,27,29-hexaene (L), was synthesized. The title complex $[Cu_2(H_{-2}L)Cl_2]\cdot 6.5H_2O$ was isolated as a blue crystal, orthorhombic, space group *Fddd*, with a = 16.4581(12), b = 32.248(2), c = 35.830(2) Å, V = 19017(2) Å^3, Z = 16, $R1$ = 0.0690, and $wR2$ = 0.1546 $[I > 2\sigma(I)]$. The protonation constants of Cu_2L were determined by potentiometric titration, and it was found that the alcoholic hydroxypropyl group of the complex Cu_2L exhibits low pK_a values of pK_{a1} = 7.31, pK_{a2} = 7.83 at 25 °C. The hydrolysis kinetics of 4-nitrophenyl acetate (NA) promoted by the title complex have also been studied. The pH-rate profile for Cu_2L gave a sigmoidal curve and showed a second-order rate constant of 0.39 ± 0.02 $M^{-1}\ s^{-1}$ in 10% CH_3CN/H_2O(v/v), which is greater than that of the dinuclear Cu^{II} complex formed by a hexaaza macrocycle without pendants. The reason for the higher catalytic activity of the title complex is discussed. We found that the volume of nucleophile RO^- can effect the hydrolysis of the carboxy ester, the nucleophilicity of RO^- and the Lewis acidity of the metal macrocycle also affect the carboxy ester hydrolysis.

Introduction

Metal hydroxides and metal alkoxides play important roles in hydrolytic metalloenzymes. Metal hydroxides have been implicated in the carbonic anhydrase catalyzed hydration of carbon dioxide[1] as well as in the carboxypeptidase-catalyzed hydrolysis of amides.[2] The role of the metal ions in the activation of proximate alcoholic OH groups is well documented in natural metalloenzymes. The key role of the metal atom in promoting hydrolysis appears to be the intramolecular delivery of the nucleophile. By virtue of its Lewis acidity, the metal ion reduces the pK_a of a coordinated water molecule, thereby producing a good nucleophile at neutral pH. In addition, the hydration sphere normally associated with water molecules in bulk solution does not interfere with nucleophilic attack in an inner-sphere reaction.[3]

Kimura's group discovered that the pendant alcohol can be deprotonated to form a Zn^{II}-bound alkoxide anion, which acts as a strong nucleophile at physiological pH.[4,5] Chin's group has investigated the reactivities and mechanisms of copper(II) hydroxide and copper(II) alkoxide for the cleavage of phosphate diesters, and discovered that the copper(II) alkoxide-promoted transesterification path is two orders of magnitude faster than the hydrolysis path, i.e., the copper(II) hydroxide path.[6] A mechanistic study of Cu^{II}-catalyzed ester hydrolysis has yielded a variety of kinetic behaviors and proposed active species. We use a macrocycle dinucleating ligand with two hydroxypropyl pendants (L in Scheme 1) to further explore the influence of the alcohol pendant on the behavior of the corresponding Cu^{II} complexes. Here, we report the crystal structure of the dinuclear Cu^{II} complex of L. The stability constants of Cu^{II} complexes of L and carboxy ester hydrolysis were also investigated. Indeed, we found that the catalytic activity of Cu_2L is higher than that of the dicopper complex with a macrocyclic ligand without hydroxy pendants. Our results indicate that the volume of nucleophile RO^-, the nucleophilicity and the Lewis acidity of the metal macrocycle may effect the carboxy ester hydrolysis.

Results and Discussion

Structure Description of $[Cu_2(H_{-2}L)Cl_2]\cdot 6.5H_2O$

The compound crystallizes in the orthorhombic system with space group *Fddd* and consists of a neutral unit of $[Cu_2(H_{-2}L)Cl_2]$ and six and a half water molecules of crystallization. Selected bond lengths and bond angles relevant to the Cu^{II} coordination sphere are given in Table 1.

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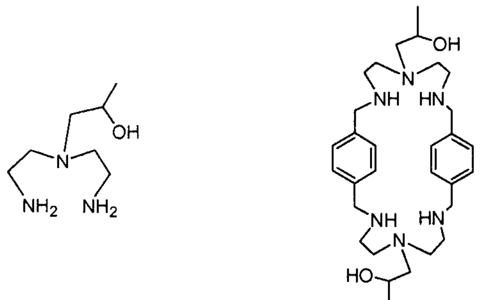


L^1 L

Scheme 1

Table 1. Select bond lengths [Å] and angles [°] for $[Cu_2(H_{-2}L)Cl_2]\cdot 6.5H_2O$

Cu1−Cl1	2.2486(15)	Cu1−N3	2.056(4)
Cu1−N1	2.018(5)	Cu1−O1	2.177(4)
Cu1−N2	2.087(4)		
N1−Cu1−Cl1	173.93(16)	N2−Cu1−O1	105.28(17)
N1−Cu1−N2	84.28(19)	N3−Cu1−Cl1	98.39(15)
N1−Cu1−N3	85.4(2)	N3−Cu1−N2	128.81(18)
N1−Cu1−O1	81.63(18)	N3−Cu1−O1	122.46(16)
N2−Cu1−Cl1	96.93(14)	O1−Cu1−Cl1	92.32(12)

As illustrated in Figure 1, each macrocycle binds two Cu^{II} ions by its diethylenetriamine moieties, which provide three nitrogen atoms to each Cu^{II} ion; the rest of the coordinated atoms are a chloride anion and an oxygen atom from the hydroxypropyl pendant. The Cu1 atom deviates 0.216 Å from the trigonal equatorial plane formed by O1, N2 and N3 towards the Cl ion with N3−Cu1−N2 = 128.81°, N3−Cu1−O1 = 122.46° and N2−Cu1−O1 = 105.28°, while the axial positions are occupied by N1 of the macrocycle and Cl1 with an N1−Cu1−Cl1 angle of 173.93°, and Cu1−N1 and Cu1−Cl1 bond lengths of 2.018 and 2.249 Å, respectively. The distortion (Δ) of the coordination polyhedron from an ideal trigonal bipyramid (Δ = 0) to a square pyramid (Δ = 1) has been calculated according to the method reported by Muetterties[7] and Galy.[8] The Δ value of 0.086 indicates that each Cu ion has a coordination geometry much closer to a trigonal bipyramid in the pentacoordinate environment. The distance between two Cu^{II} ions is 8.128 Å, which is similar to those observed in other dicopper(II) macrocyclic complexes.[9] With the center-to-center distance of 5.662 Å, the two phenyl rings form a dihedral angle of 83.3° to each other. Additionally, the two hydroxypropyl arms are located on the same sides of the plane defined by the nitrogen atoms and the phenyl groups of the macrocyclic ligand, thus ligand L in the title complex has a boat-like (*syn*) conformation.

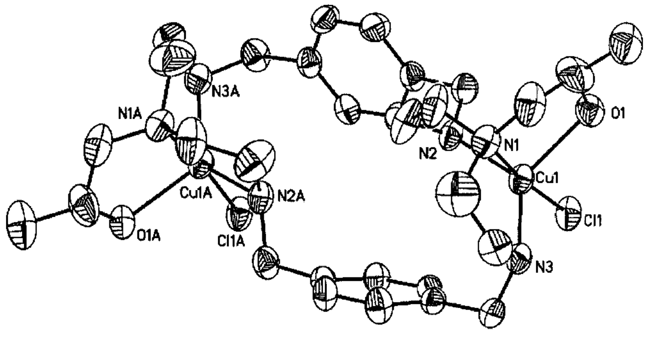


Figure 1. ORTEP drawing (30% probability ellipsoids) of $[Cu_2(H_{-2}L)Cl_2]\cdot 6.5H_2O$; hydrogen atoms and water molecules are omitted for clarity

We noticed that the Cu^{II}−O(ROH) bond length (2.177 Å) in the title complex is shorter than the Cu^{II}−O distance of 2.186 Å in the dicopper complex of a 26-membered macrocycle ligand with two hydroxyethyl groups.[9] Since shorter water oxygen−copper bonds always lead to greater acidity, and hence to lower pK_a values,[6] the discrepancy in the structure between these two Cu^{II}−macrocycle complexes may be responsible for the different deprotonation behavior.

Complexation Constants of L with Cu^{II}

The complexation constants of the species formed by L and Cu^{II} in a 1:2 [L/Cu^{II}] system with I = 0.10 M (NaNO$_3$) in aqueous solution at 298 K have been determined by potentiometric titration (6 equiv. of HCl added to acidify the pure ligand before titration). As shown in Figure S1 (see Supporting Information), when 2 equiv. of Cu^{II} are added to the solution, the titration reveals two inflexions at a = 6 and a = 8 (a is the number of mol of NaOH added per mol of ligand), respectively. The inflexion at a = 6 corresponds to the completion of the neutralization of the six ammonium ions in the diethylenetramine moieties, which are easily deprotonated to form dinuclear complexes. The inflection at a = 8 indicates the further deprotonation of two titratable protons from two hydroxypropyl pendants bound to the Cu^{II}.

To rationalize the model selection and data process, several different analysis processes were conducted and evaluated. In the first case, the deprotonated species were set to be the dinuclear complexes of $[Cu_2H_{-1}L]^{3+}$ and $[Cu_2H_{-2}L]^{2+}$. In the second case, the deprotonated species were set for both dinuclear and mononuclear complexes; i.e., the species CuL, CuHL, CuH$_2$L, Cu$_2$L, $[Cu_2H_{-1}L]^{3+}$, $[Cu_2H_{-2}L]^{2+}$ and $[CuH_{-1}L]^{+}$ were included. In the third case, all possible deprotonated species and hydroxide species were included. In the third case, reasonable results were given by BEST, with a σ-fit value optimized to be 0.028. However, in other cases, the σ-fit values are all larger than 0.2. Thus, we chose the third case as the most rational one.

Figure 2 displays the species distribution as a function of pH at a total Cu^{II} concentration of 2 mM and a total L concentration of 1 mM at 25 °C. As shown in Figure 2, $[CuH_2L]^{4+}$, $[Cu_2L]^{4+}$, $[Cu_2H_{-1}L]^{3+}$, $[Cu_2H_{-2}L]^{2+}$, $[Cu_2H_{-2}L(OH)]^{+}$ and $[Cu_2H_{-2}L(OH)_2]$ exist as the major Cu^{II} complex species under the employed experiment conditions, and the other species were less than 1 ppm in concentration. The dinuclear complex $[Cu_2L]^{4+}$ is the predominant species over the pH range of 5−6.5. The monodeprotonated $[Cu_2H_{-1}L]^{3+}$ has a favourable pH of 7.4, above which it is gradually converted into $[Cu_2H_{-2}L]^{2+}$, $[Cu_2H_{-2}L(OH)]^{+}$, $[Cu_2H_{-2}L(OH)_2]$; $[Cu_2H_{-2}L]^{2+}$ predominates at pH values of about 8.5, and this is in accordance with the X-ray crystal structure analysis. To illustrate the process clearly, the stepwise complexation of L with Cu^{II} via these species is shown in Scheme 2. ES-MS data (shown in Figure 3a) for the complex in 10% CH_3CN/H_2O (v/v) at a pH of about 6.0 in aqueous solution evidence the stable existence of $[Cu_2LCl_2]^{2+}$ (m/z = 362.1) and $[Cu_2LCl_3]^{+}$ (m/z = 759.0). The stable existence of these species indicates that the dinuclear complex $[Cu_2L]^{4+}$ is the predominant species at a pH of about 6.0.

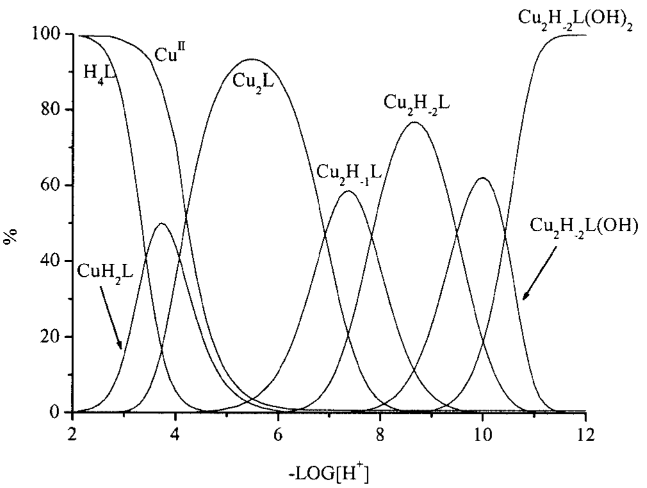
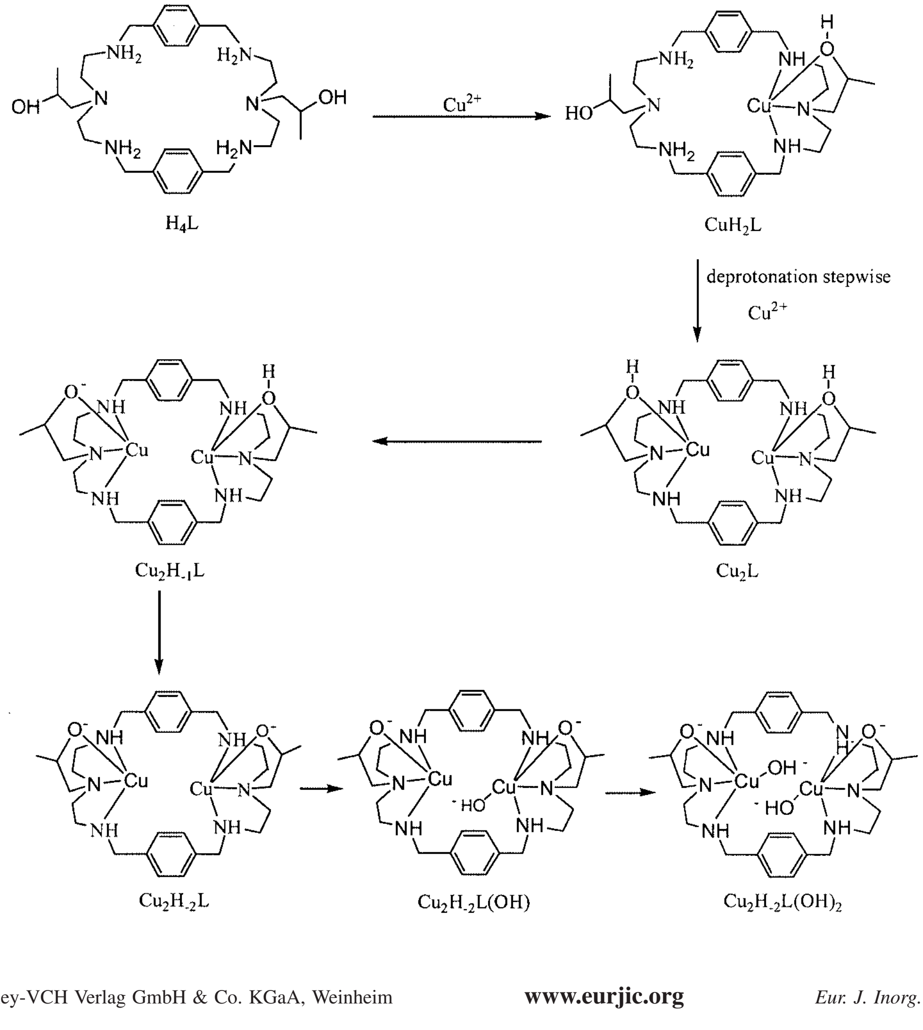


Figure 2. Species distribution graph as a function of pH for the 1 mm L + 2 mm Cu^{II} system at 25 °C and I = 0.10 m ($NaNO_3$)

The program BEST was used to analyze the titration data, and the obtained log K and the deprotonation constant pK_a for the Cu^{II} complexes with L at 25 °C are listed in Table 2. The most significant finding from Table 2 is the rather facile deprotonation of the alcoholic function with pK_a = 7.31 at 25 °C, which is lower than that found by Kimura et al.[4] for the Zn^{II} complex of 1-(2-hydroxyethyl)-1,5,9-triazacyclodecane (pK_a = 7.40 at 25 °C) and a dicopper complex of a 26-membered macrocyclic ligand with two hydroxyethyl pendants (pK_a = 7.60).[9] This lower pK_a value implies that the title complex is a strong enough nucleophile hydroxyethyl pendants (pK_a = 7.60).[9] This lower pK_a value implies that the title complex is a strong enough nucleophile to attack an ester bond in catalytic hydrolysis.

Hydrolysis of 4-Nitrophenyl Acetate

The reactivity of the dinuclear complex toward the hydrolysis of phosphate ester or carboxy ester has also been checked. Since phospho monoesters undergo slow hydrolysis with the complex, we only describe the hydrolytic cleav-



Scheme 2

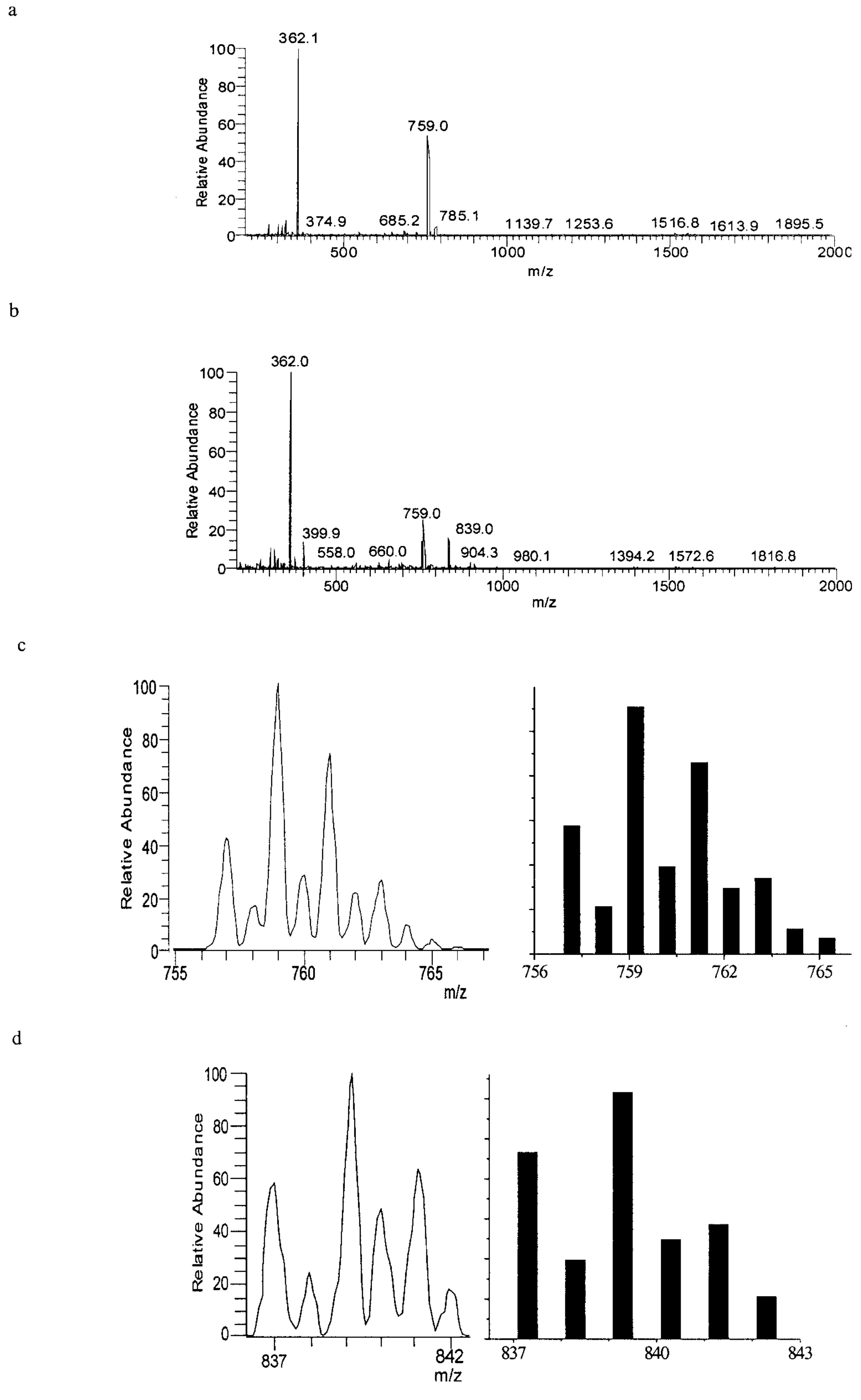


Figure 3. (a) Electrospray mass spectrum of the complex at pH ≈ 6 and 10% CH_3CN/H_2O (v/v) solution; the peak at m/z = 362.1 is assigned to $[Cu_2LCl_2]^{2+}$, m/z = 759.0 is assigned to $[Cu_2LCl_3]^+$; (b) electrospray mass spectrum for the mixture of Cu_2L/NA = 1:1 at pH ≈ 6 and 10% CH_3CN/H_2O (v/v) solution; the peak at m/z = 836.9 is assigned to $[IaH_{-2}Cl(CH_3OH)_2]^+$; (c) comparison of the observed (traces) and calculated (bars) isotopic distributions for the peak at m/z = 759.0 in the ES-MS data of the complex; (d) comparison of the observed (traces) and calculated (bars) isotopic distributions for the peak at m/z = 836.9 in the ES-MS data for the mixture of equimolecular Cu_2L and NA

Table 2. Complexation constants and deprotonation constants for Cu^{II} complexes with $I = 0.1$ M ($NaNO_3$) at 25 °C

Symbol	Equilibrium quotient	log*K*
log*K*(CuL)	[CuL]/[Cu][L]	15.17 (0.03)
log*K*(CuHL)	[CuHL]/[CuL][H]	8.57 (0.02)
log*K*(CuH_2L)	[CuH_2L]/[CuHL][H]	5.76 (0.03)
log*K*(Cu_2L)	[Cu_2L]/[CuL][Cu]	6.83 (0.025)
pK_{a1}	[$Cu_2H_{-1}L$][H]/[Cu_2L]	7.31 (0.05)
pK_{a2}	[$Cu_2H_{-2}L$][H]/[$Cu_2H_{-1}L$]	7.83 (0.06)
pK_{a3}	[$Cu_2H_{-2}L(OH)$][H]/[$Cu_2H_{-2}L$]	9.48 (0.03)
pK_{a4}	[$Cu_2H_{-2}L(OH)_2$][H]/[$Cu_2H_{-2}L(OH)$]	10.56 (0.02)

age of 4-nitrophenyl acetate (NA). The activity of the dinuclear species has been investigated by monitoring the hydrolysis of NA (0.4−1.0 mM) promoted by Cu_2L at different concentrations (0.5−2.0 mM) at pH = 7.3−9.3, as described in the Exp. Sect. The spontaneous hydrolysis of NA by buffered solution was subtracted from the Cu complex promoted hydrolysis. A second-order kinetics of Cu_2L promotion NA hydrolysis was observed at different pH values. The initial rate constant, k_{in}, observed rate constant, k_{obs}, the spontaneous hydrolysis constant, v_{buffer}, and second-order constant, k_{cat}, are defined as follows:

$$v_{total} = v_{Cu} + v_{buffer} = k_{in}[NA]$$
$$v_{buffer} = (k_{OH}{}^{-}[OH^{-}] + ...)\cdot[NA]$$
$$v_{Cu} = k_{NA}\cdot[Cu^{II}complex]_{total}\cdot[NA] = k_{cat}\cdot[Cu_2H_{-2}L][NA]$$

The first-order constant k_{obs} [s^{-1}] is calculated from the slope of the straight line v_{Cu} vs. [NA]. The plot of k_{obs} vs. [Cu^{II} complexes]$_{total}$ gives a straight line with the slope denoted as k_{cat} ($M^{-1}s^{-1}$).[6] The second-order rate constant k_{cat} for Cu_2L is 0.39 $M^{-1}s^{-1}$ at pH = 9.5 (shown in Figure 4), where [$Cu_2H_{-2}L$]$^{2+}$ is the main species in aqueous solution.

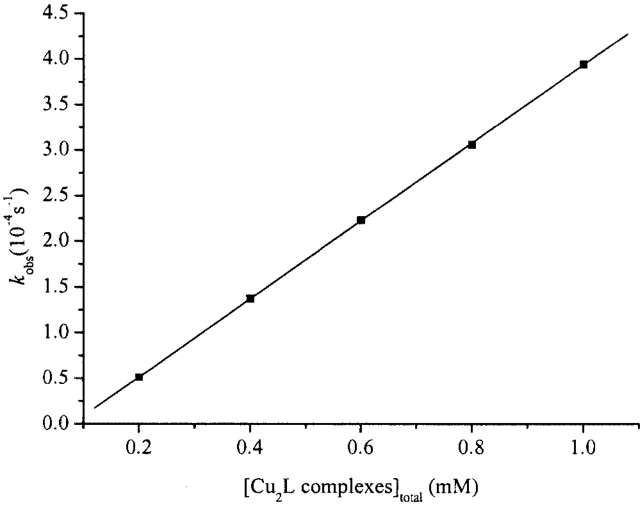


Figure 4. Dependence of k_{obs} on [total Cu^{II} complexes] at pH = 0 9.5, ionic strength 0.1 M ($NaNO_3$), and at 25 °C in the presence of 10% CH_3CN/H_2O (v/v); correlation coefficient > 0.99

To examine whether the whole process is a catalytic hydrolysis or transesterification, we followed the release of NP up to more than one cycle with the NA concentration set at 6.0 mM and the Cu^{II} complex concentration at only 0.2 mM in buffer solution (pH = 9.0) by monitoring the absorbance at 400 nm at 25 °C. The resulting v_{total} vs. time curve reveals that the rate constant for Cu_2L is identical to the initial rate constant determined above. Thus, the mechanism is catalytic hydrolysis. The second-order constants k_{cat} are plotted as a function of pH, and the resulting sigmoidal curve is shown in Figure 5. This curve is characteristic of a kinetic process controlled by an acid/base equilibrium with an inflexion at pH ≈ 8.75, and, as shown in Figure 2, [$CuH_{-2}L$]$^{2+}$ is the major species in aqueous solution at pH ≈ 8.7. Therefore, the main promoter species is proposed to be the deprotonated species [$CuH_{-2}L$]$^{2+}$. Different from the reported dicopper complex of a 26-membered macrocyclic ligand with two hydroxyethyl pendants,[9] the maximum value of k_{cat} for Cu_2L appears at pH ≈ 9.0 because the concentration of [$CuH_{-2}L$]$^{2+}$ species decreases significantly when the solution's pH is higher than 9.0. As with the reported dicopper complex,[9] the value of k_{cat} increases further when the solution's pH is higher than 9.5 because the doubly deprotonated species is still the major component.

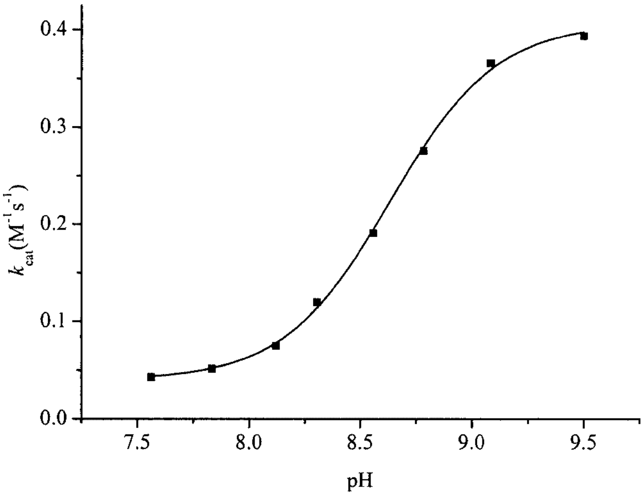
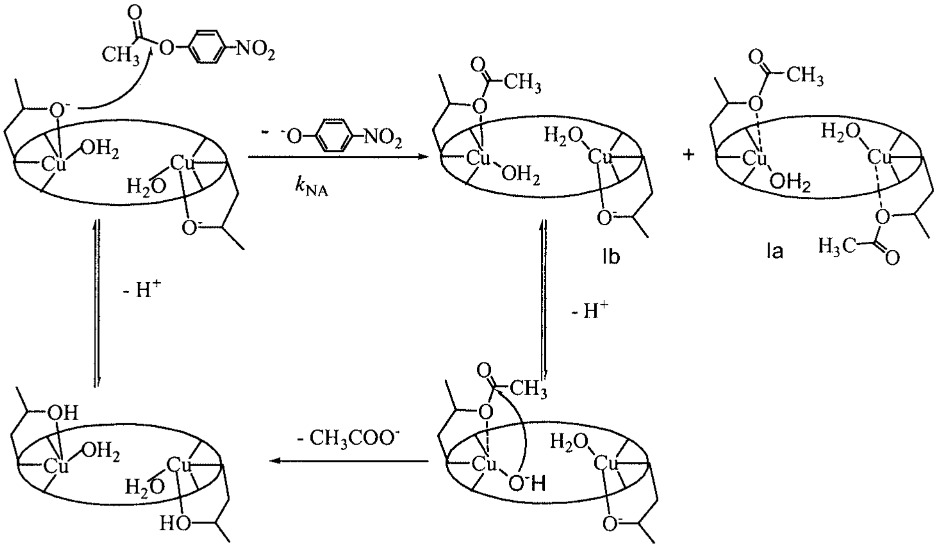


Figure 5. pH/rate profile for the second-order rate constant of NA hydrolysis with the Cu_2L complex at 25 °C and $I = 0.1$ M ($NaNO_3$) in 10% CH_3CN/H_2O (v/v)

In the course of catalytic NA hydrolysis, we suspected that a transient "acetyl intermediate" is formed, which then disappears very rapidly to give the final product acetate anion and Cu_2L. At pH values of 6 or lower, the intermediate can exist in aqueous solution for long time and it will disappear quickly when the pH of the solution is adjusted to 8.8.[10] A non-buffered solution (pH ≈ 6) of Cu_2L/NA = 1:1 in 10% CH_3CN/H_2O (v/v) was analyzed by ES-MS (shown in Figure 3b). When compared to Figure 3a, a new peak at *m*/*z* = 839.0 appears after the addition of NA and this peak is assigned to the intermediate [**Ia**$H_{-2}Cl(CH_3OH)$]$^{+}$ (Scheme 3). On this basis, we propose



Scheme 3

the overall possible catalytic reaction depicted in Scheme 3, with the initial formation of an "acyl intermediate" being the slowest step. The formation of a similar intermediate was also observed by the groups of Kimura, Bencini and ourselves in NA hydrolysis catalyzed by Zn^{II} complexes.[4,10–12]

Comparing the kinetic data for Cu_2L and other dicopper(II) macrocyclic complexes, we found that the rate constant for Cu_2L is similar to that of a dicopper complex of a 26-membered macrocyclic ligand with two hydroxyethyl pendants (0.41 $\text{M}^{-1}\text{s}^{-1}$), and approximately 14 times higher than the Cu^{II} complex of the macrocyclic ligand without hydroxy pendants (0.027 $\text{M}^{-1}\text{s}^{-1}$).[9] These results suggest that a Cu^{II}-bound alkoxide is a better nucleophile than a Cu^{II}-bound hydroxide. The similar hydrolysis rate constants of Cu_2L and the reported dicopper hydroxyethyl macrocycle complex[9] may be related to this. The substitution of 2-hydroxypropyl for hydroxyethyl increases the nucleophilicity of the metal macrocycle and thus increases the hydrolysis rate constant, while the addition of a methyl group can increase the nucleophilic reaction's steric constraint and does not benefit the nucleophilic attack. The other reason may be the Lewis acidity of the metal complex. This trend of increasing Lewis acidity with increasing macrocycle size can be observed through the comparison of the pK_a of Cu_2L ($pK_{a1} = 7.31$, $pK_{a2} = 7.83$) and a reported dicopper complex of a hydroxyethyl macrocycle ($pK_{a1} = 7.60$, $pK_{a2} = 8.07$).[9] Since Cu_2L is a stronger Lewis acid than the Cu^{II} hydroxyethyl macrocycle complex, Cu_2L is expected to activate the coordinated carboxy ester toward nucleophilic attack to a greater degree than the other complex, although as the metal complex becomes a stronger Lewis acid, the nucleophilicity of the coordinated hydroxide decreases.

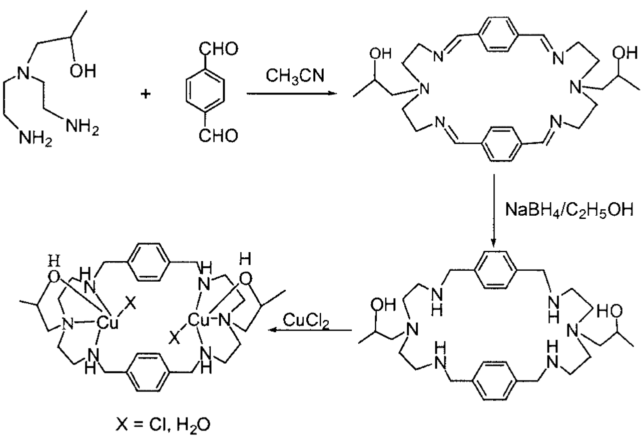
Conclusions

A new dinuclear Cu^{II} complex of a macrocyclic ligand with two 2-hydroxypropyl pendants has been synthesized. X-ray crystal analysis reveals that the two pendant alcohol groups are deprotonated to bind two Cu^{II} ions, which both adopt a trigonal-bipyramid geometry with a Cu···Cu distance of 8.129 Å. The solution complexation studied by potentiometric titration on the Cu^{II} complexes of L has revealed that $[Cu_2H_{-2}L]^{2+}$ is the major species when the pH of an aqueous solution is around 8.7, which is in accordance with the X-ray crystal analysis result. The 4-nitrophenyl acetate hydrolysis promoted by Cu_2L shows a second-order rate constant of 0.39 $\text{M}^{-1}\text{s}^{-1}$, which is approximately 14 times higher than that of the dicopper macrocycle complex without hydroxy pendants, while it is similar to that of a similar Cu^{II} complex of a macrocycle with hydroxyethyl pendants, which suggests that a Cu^{II}-bound alkoxide is a better nucleophile than a Cu^{II}-bound hydroxide. In spite of the volume of RO^-, the nucleophilicity and the Lewis acidity of the metal macrocycle can also effect the carboxy ester hydrolysis.

Experimental Section

Instrumentation and Materials: C, H and N analyses were performed with a Perkin−Elmer 240C elemental analyzer. ES mass spectra were obtained with a Finnigan MAT LCQ ES mass spectrometer with an *m/z* range of 2000. A CH_3OH/water (1:1) mixture was used as the solvent, and the concentration used in the experiment was 1.0 µmol/L. 1-[Bis(2-aminoethyl)amino]-2-propanol (L^1, Scheme 1) was prepared as described previously.[13] The other reagents were of analytical grade from commercial sources and were used without further purification. The synthesis of L and its Cu^{II}

complex is shown in Scheme 4. L was synthesized as a mixture of diastereomers by $NaBH_4$ reduction of the corresponding Schiff base obtained from the [2 + 2] condensation between 1-[bis(2-aminoethyl)amino]-2-propanol (L^1) and terephthaldehyde similar to the reported procedures,[9] and then the Cu_2L complex was synthesized by reaction of L with $CuCl_2$.



Scheme 4

Preparation of the Dinuclear Copper Complex $[Cu_2(H_{-2}L)Cl_2]·6.5H_2O$: A solution of L (0.0512 g, 0.10 mmol) in H_2O (2 mL) was added dropwise with stirring to an aqueous solution (2 mL) containing $CuCl_2·2H_2O$ (0.0341 g, 0.20 mmol), at 25 °C, to give a blue solution (pH ≈ 8.5). After being stirred for 2 h, the reaction mixture was filtered. Blue polyhedral crystals were obtained by slow evaporation of the solvents from the filtered solution for several days at ambient temperature. $[Cu(H_{-2}L)Cl_2]·6.5H_2O$ (839.83): calcd. C 43.09, H 6.94, N 10.13; found C 43.07, H 6.82, N 10.05.

X-ray Crystallographic Study of $[Cu_2(H_{-2}L)Cl_2]·6.5H_2O$: Diffraction data for the crystal were collected with a Siemens SMART CCD system equipped with monochromated Mo-K_α radiation (λ = 0.71073 Å) at room temperature in the range of $2.20° \le \theta \le 25.00°$. Data integration and empirical absorption corrections were carried out with the SAINT[14] and SADABS[15] programs. The structure was solved by direct methods (SHELXS 97) and refined on F^2 by full-matrix least-squares techniques (SHELXL 97).[16] All the non-H atoms were refined anisotropically. Hydrogen atoms were introduced in calculated positions and treated as riding atoms using the SHELXL-97 default parameters. All of the crystalline water molecules were found to be disordered and the site occupancy factors (s.o.f.s) for these oxygen atoms (O1W, O2W, O3W, O1W′, O2W′, O3W′, O4W, O5W, O6W) are 0.5, 0.25, 0.25, 0.5, 0.5, 0.25, 0.5, 0.5 and 0.25, respectively. The O5W atom is located on the twofold axis. The crystallographic data and details of the data collection are summarized in Table 3. CCDC-210142 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Table 3. Summary of crystal data, data collection and refinement for $[Cu_2(H_{-2}L)Cl_2]·6.5H_2O$

Empirical formula	$C_{30}H_{61}Cl_2Cu_2N_6O_{8.5}$
Formula mass	839.83
λ [Å]	0.71073
Crystal system	orthorhombic
Space group	*Fddd*
a [Å]	16.4581(12)
b [Å]	32.248(2)
c [Å]	35.830(2)
V [Å^3]	19017(2)
Z	16
ρ [g cm^{-1}]	1.173
Measured reflections	21787
Unique reflections	4034 (R_{int} = 0.0613)
Observed reflections [$I > 2\sigma(I)$]	2288
μ [mm^{-1}]	1.051
Goodness-of-fit on F^2	1.026
$R1$,[a] $wR2$[b] [$I > 2\sigma(I)$]	0.0694, 0.1566
Largest difference peak/hole [e Å^{-3}]	0.578/−0.339

[a] $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.
[b] $w = 1/[(F_o^2)^2 + (0.0354P)^2 + 0.0000P]$, where $P = (F_o^2 + 2F_c^2)/3$.

Potentiometric Measurements: Potentiometric experiments were conducted with an Orion 91−04 glass combination pH electrode and an Orion microprocessor analyzer/901 at 25 °C. All solutions were carefully protected from air by a stream of humidified nitrogen. Standard NaOH (0.1 M, CO_2 free) was added by a spiral micro-injector. The $CuCl_2$ solution was calibrated by standard EDTA, and doubly distilled water with a pH of about 6 was used. The temperature of the cell was controlled by a thermostat. K_w was chosen as 13.69 for 25 °C, 0.1 M $NaNO_3$ was contained to adjust the ionic strength to 0.1 M. Solutions containing 1 mM L with or without 2 mM $CuCl_2·2H_2O$ were titrated to pH > 11. The complexation and protonation constants were calculated using the program BEST.[17] All data represent the average of at least two independent experiments and all the sigma fit values have been reduced to less than 0.03 after optimization.

Kinetic Studies: The hydrolysis rate of 4-nitrophenyl acetate (NA) was measured by an initial slope method by monitoring the increase in the 400-nm absorption of the released 4-nitrophenolate in 10% CH_3CN/H_2O (v/v) aqueous solution at 25.0 ± 0.5 °C with a UV-3100 spectrophotometer by using the procedures reported previously.[9,10] Buffered solutions containing Tris buffer (pH = 7.3−9.0) or CHES buffer (pH = 9.3−9.6) were used, and the ionic strength was adjusted to 0.10 M with 1.0 M $NaNO_3$. The initial rate determination was carried out according to the typical procedure previously reported by Kimura and ourselves.[4,5,9,10,18] NA and Cu^{II} complex were mixed in the buffered solution, the UV absorption increase at 400 nm was recorded immediately and monitored generally until 2% formation of 4-nitrophenolate. The observed first-order rate constant k_{obs} [s^{-1}] was calculated from the decay slope of 4-nitrophenolate release rate vs. [NA] and the slope of k_{obs} vs. [Cu^{II} complex]$_{total}$ was denoted as the second-order rate constant k_{NA} [M^{-1}s^{-1}]. All the experiments were conducted at least twice, and the tabulated data represent the average of these experiments.

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